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THE EYE STRUCTURE OF THE BIOLUMINESCENT FIREWORM OF BERMUDA, *ODONTOSYLLIS ENOPLA*

JEROME J. WOLKEN[1] AND ROBERT G. FLORIDA[2]

[1]*Carnegie-Mellon University, Mellon Institute, Department of Biological Sciences, 4400 Fifth Avenue, Pittsburgh, Pennsylvania 15213 and* [2]*Magee-Womens Hospital, University of Pittsburgh Medical Center, Pittsburgh, Pennsylvania 15260*

Abstract

The polychaete annelid, *Odontosyllis enopla* Verril of the family syllidae in the Bermudas, possesses a lunar periodicity and bioluminesces during the mating period. *Odontosyllis* has four eyes arranged so that two are located on each side of its head. The eyes are situated on lobes that have some degree of movement. Light sensitivity is maximum for illumination along the optical axis, which is different for the anterior and posterior pairs. The eye resides in a cavity formed by the pigment granules, and is structured of a lens, photoreceptor cells, the retina, and pigment granules. The retinal photoreceptors approach and surround the lens. The photoreceptors are formed of membranous processes, lamellae, and are similar in structure to the rhabdomeric photoreceptors of arthropods, cephalopod mollusc eyes, and the retinal rod outer segment of vertebrate eyes. The lens is a spheroidal body composed of cells. Closely associated with the lens are rods (tubes) about 60 nm in diameter arranged in linear arrays, suggesting that they are fiber optic bundles. The function of the fiber optic system could be to detect the direction of the bioluminescent light and to maximize the light-collecting ability of the eye.

Introduction

In the search for evolutionary clues in the development of the eye and vision, a comparative study of the eyes in a variety of invertebrate marine animals has been under continuous investigation (Wolken and Florida, 1969; Wolken, 1971, 1974, 1975). Among invertebrates, the arthropods and molluscs, evolved imaging eyes, and the question arose whether among annelids image forming eyes also evolved.

The eye structure of polychaete annelid was described some time ago by Greef (1877), Andrews (1892), and later by Hesse (1899). More recently Hermans and Eakin (1974) investigated by electron microscopy the fine structure of the eye on an alciopid, *Vanadis tangensis*. This was followed by Wald and Rayport (1977), who demonstrated that the alciopids *Torrea candida,* a surface worm, and *Vanadis,* found in the deep sea, possess image resolving eyes. To investigate the visual system of *Odontosyllis enopla* Verril, of the family syllidae, known as the "fireworm" of Bermuda, was of interest to us due to the early studies of Galloway and Welch (1911). *Odontosyllis* possesses a lunar periodicity throughout the year. The mating period continues from one to three days or more after the full moon, occuring precisely 50–55 minutes after sunset (Markert *et al.,* 1961). The females luminesce at the surface, attracting the males, who also luminesce (Huntsman, 1948). The bioluminescent color observed in its natural environment is bluish-green, but in the laboratory upon agitation, the

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live worms bioluminesce a bright green. The bioluminescent substance has been chemically identified as a luciferin system (Shimomura *et al.,* 1963; Trainor, 1979).

The response of *Odontosyllis* eyes to light stimuli of various wavelengths was determined by recording (ERG) electroretinogram (Wilkens and Wolken, 1981). The visual spectral peak response was around 510–520 nm, which coincides with the bioluminescent luciferin system emission peak of around 507–516 nm (Shimomura *et al.,* 1963; Nicol, 1978). This suggests that the response is a visual one for sensing the bioluminescent light. As a result of these studies we became interested in examining in greater detail how the eye of *Odontosyllis enopla* is structured for light reception.

Materials and Methods

The Bermuda fireworms *Odontosyllis enopla* were collected during the months of July and August (1979–1981) in regions of shallow grass beds lining the shore of Whalebone Bay on the north shore of Bermuda. Both female and male worms were collected one hour after sunset and from one to five days after the full moon, by netting the worms at the surface during their bioluminescent mating displays.

For microscopy, both whole worms and the heads of worms were immediately fixed, some in 2.5% gluteraldehyde, pH 7.4, and others in Karnovsky's fixative, buffered with Millonig's phosphate buffer, pH 7.4. The fixed *Odontosyllis* were then refrigerated (4°C) until they were further processed for microscopic examination.

For electron microscopy, the fixed specimens were post fixed in 2% OsO_4 and the entire block stained with 2% aqueous uranyl acetate. All excess tissue was removed, and the organisms were dehydrated through a graded series of alcohols and finally in propylene oxide. They were placed in Mollenaur's number 2 formula for Epon-Aralidite and embedded in flat molds, in order to orient the eyes properly for sectioning.

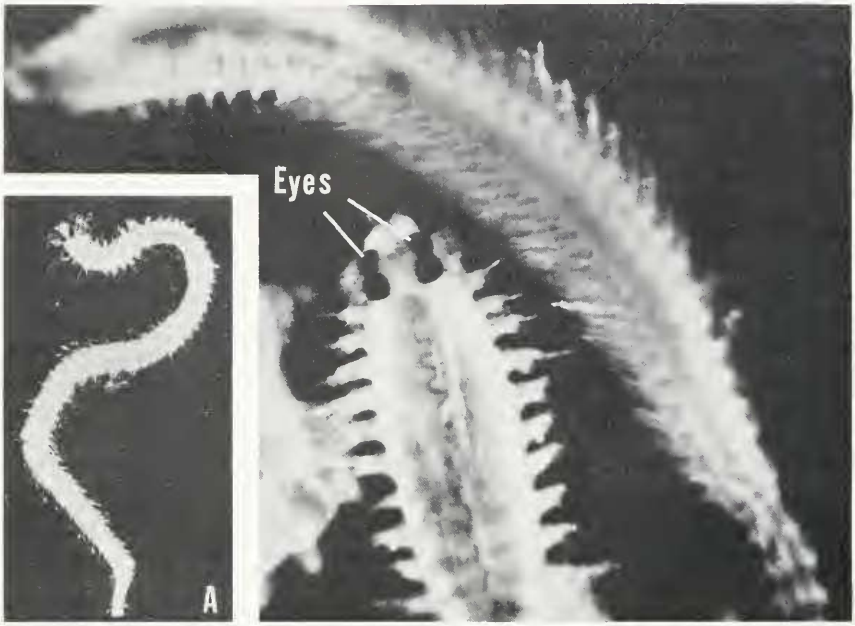
One micron sections were cut with glass knives on an LKB-Huxley Ultramicrotome. These sections were stained with methylene blue and examined by light microscopy. For transmission electron microscopy, the best fixed material was selected. Thin sections were then cut with a diamond knife on the Reichert Om-U2 Ultramicrotome. These sections were then double stained with 5% aqueous uranyl acetate for 20 minutes and then with Reynold's lead citrate for 5 minutes. The sections were scanned using the Philips Em-300 electron microscope, and various areas of the eye were photographed.

For scanning electron microscopy, the specimens were treated identically to those for transmission electron microscopy. The organisms were post fixed in OsO_4 and then dehydrated through a graded alcohol series to one hundred percent ethyl alcohol. The fixed and dehydrated organisms were dried in a Polaron Critical Point Dryer, mounted on studs, and coated in the Polaron E5100 sputter coater. The organisms were examined and photographed using the AMR-1000 scanning electron microscope in the Department of Biological Science, University of Pittsburgh.

To extract the visual pigment from the *Odontosyllis* eyes, 500 heads were dissected from the worms under red light in a cold room and were freeze dried. After thawing, the extraneous material was centrifuged away. The resulting material was then extracted with 1–2% aqueous digitonin in phosphate buffer, pH 7.5, in the dark at room temperature over night. The digitonin extract was then fractionated using a sucrose gradient density method followed by centrifugation and then ultracentrifugation (30,000 rpm for 1 hour). This resulted in a pink-colored layer that was removed from the centrifuge tube; its absorption spectrum was obtained in a Cary-14 Spectrophotometer.

RESULTS

Odontosyllis has four eyes arranged so that two pairs are located adjacent to each other on the dorsal surface of the head (Fig. 1). The eyes are on protruding lobes that have some movement (Fig. 2). There were individual differences in the locations



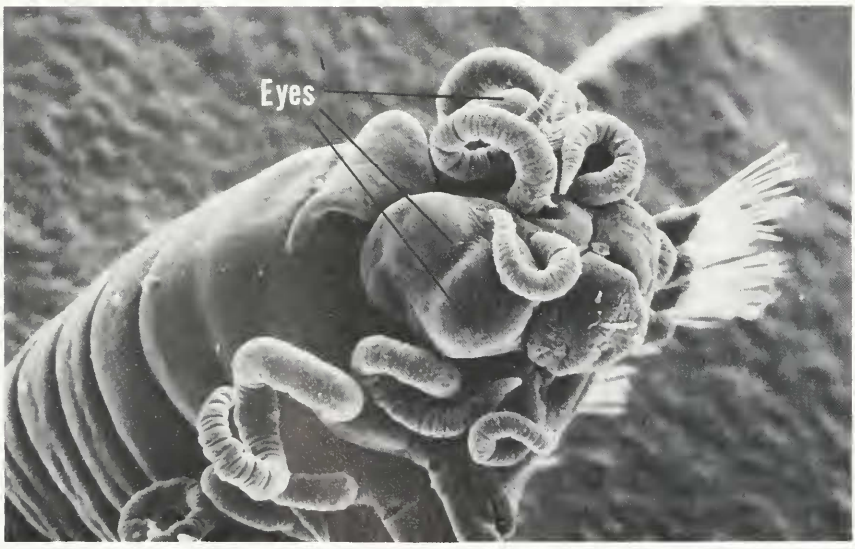


FIGURE 1. (Top) *Odontosyllis enopla* Verril. Live male and part of a female showing the two pairs of eyes. 13×. A. Live female (22 mm long, 1.5 mm in diameter). Photographed in laboratory with dark field illumination. 2.5×.

FIGURE 2. (Bottom) Scanning electron microscopy (SEM) of head area of *Odontosyllis*, showing lobes in which the two eyes are located. 106×.

of the eyes, as well as in their relative size. The eyes of the males are larger than the eyes of the females. The eyes seemed to change in size and pigmentation with time in the laboratory. In females collected during the breeding period, the eyes were heavily pigmented, with the anterior eyes being larger than the posterior ones (Figs. 3, 4). However, after 7–10 days in the laboratory, the anterior eyes had decreased in size and were more nearly the size of the posterior eyes, or smaller. In addition, both pairs of eyes had become less heavily pigmented, giving rise to the appearance of some of the inner structures of the eye.

The front of the eye is almost completely covered by the worm's cuticle, and each eye has a rounded exterior with a relatively small opening. The lens is located behind the opening. The position of the lens can only be distinguished from the rest of the darkly pigmented eye with the aid of a stereomicroscope. The lens is a spheroidal gelatinous body lying in the cavity formed by the pigmented cup (Fig. 5). From the

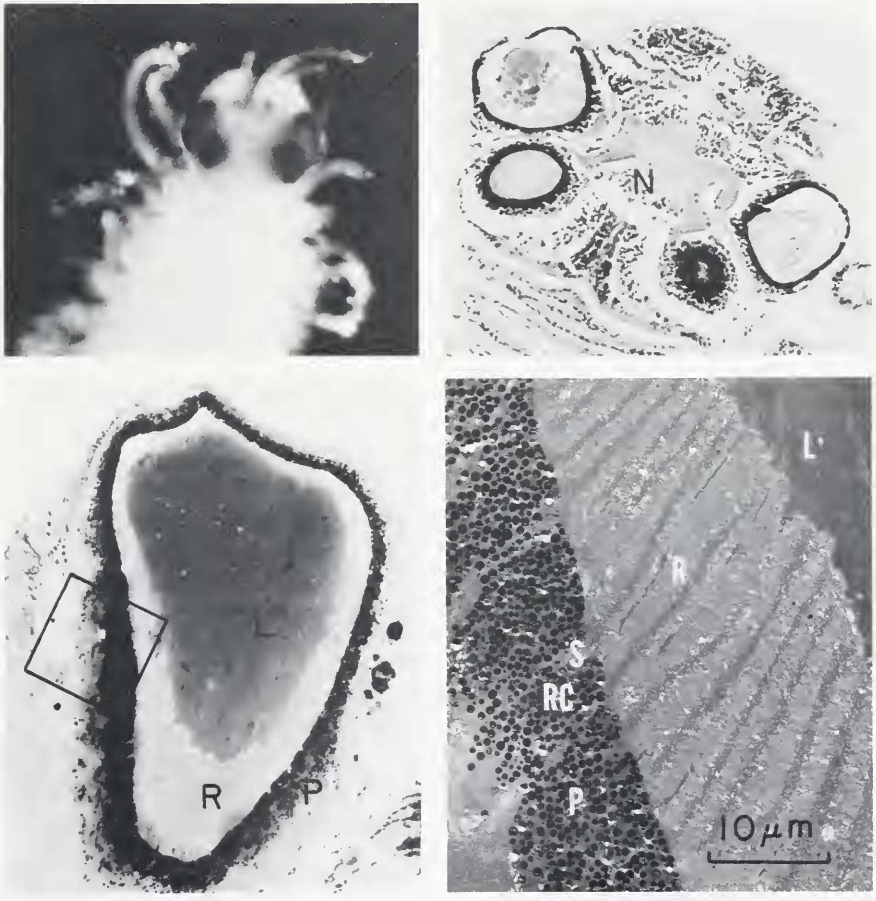


FIGURE 3. (Top left) Preserved female worm, with the two eyes on each side of the head anterior segments. 17×.

FIGURE 4. (Top right) Cross-section of a cut through the head, histologically stained, showing the two eyes on each side of the head and their relative size. N, region of neural structures. 92×.

FIGURE 5. (Bottom left) Section through the eye, showing the lens (L), photoreceptors (R), and pigment granules (P) that surround the eye. EM. 306×.

FIGURE 6. (Bottom right) Higher magnification of boxed area in Figure 5, showing part of the lens (L), photoreceptors (R) and pigment granules (P), receptor cells (RC), supporting cells (S). EM.

contour of the eyes and the location of the lens, the optical axis of the anterior eyes appears to be directed forward and lateral whereas the axis of the posterior eyes appears to be directed dorsally, with a slight forward-lateral bias (Wilkens and Wolken, 1981). A cross-section cut through the eye shows the lens, photoreceptors, and pigment granules (Figs. 4–6). Microscopic examination of sections of the lens show that it is composed of cells. Electron microscopy of the lens indicated that within the lens area there are rods or tubes about 60 nm in diameter that are arranged in a uniform linear array (Figs. 7–9).

The retinal photoreceptors surround the lens and appear to be in contact with the lens (Figs. 5, 6). The retinal photoreceptors vary in length and measure up to 45

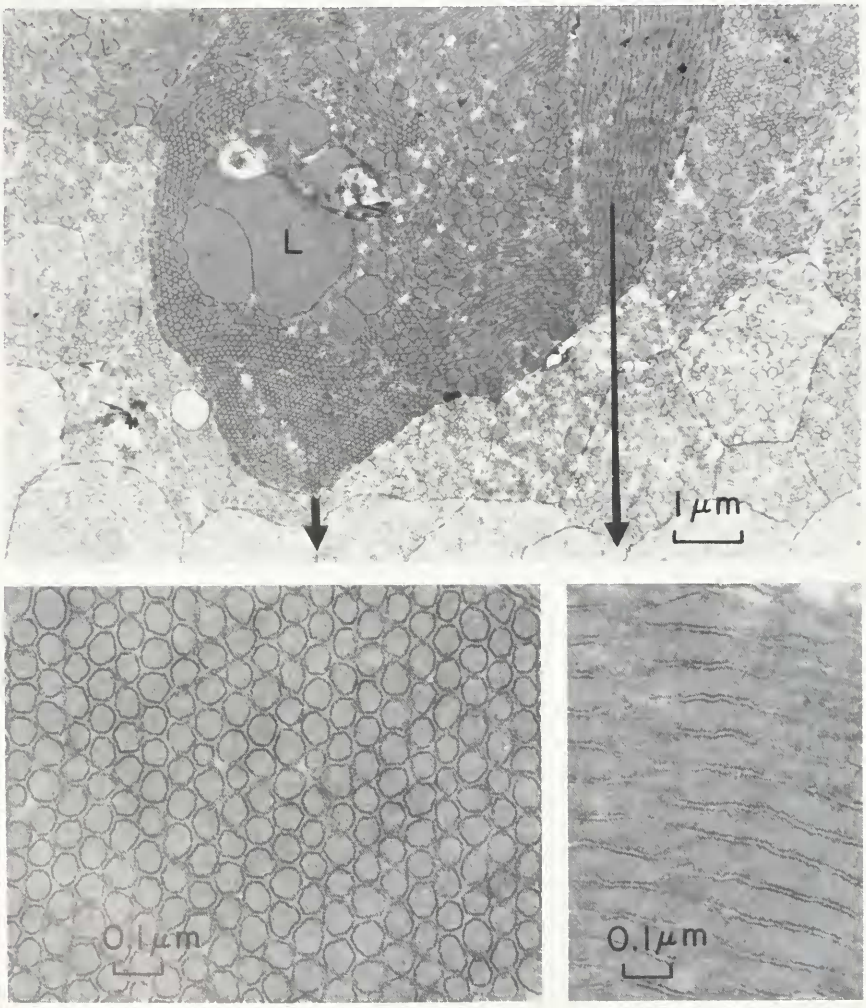


FIGURE 7. (Top) The lens, cells, and tubular structures in the lens. Compare shape of lens to Figure 5.

FIGURE 8. (Bottom left) The tubules, higher magnification of Figure 7, in cross-section arranged in linear arrays in the lens. EM.

FIGURE 9. (Bottom right) The lens, higher magnification of Figure 8, longitudinal section of the tubules.

μm and are about 1–2 μm in width (Fig. 10). The retinal photoreceptors are formed by membranous processes into lamellae that are about 70 nm in thickness (Fig. 10). The lamellae are aligned with the optical path and lie at right angles to the light path.

The retinal cells are separated by an extension of "grey" cell material and by mitochondria. The "grey" cells are located at the posterior end of the photoreceptors and are adjacent to them (Fig. 6). They contain the bulk of the pigment granules and form the inner part of the enclosure of the eye. There are openings (appearing circular) through or between these "grey" cells in which mitochondria are observed that are continuous with the regions that separate the photoreceptors. Myelinated bodies appear in these cells and in the cells adjoining them. There are also neural structures in the cells lying behind the photoreceptors which connect directly to the "grey" cells and to supporting cells that penetrate into this cellular region. Formalin fixed histological sections stained with hematoxylin and eosin indicated that neural fibers were located in the region (Fig. 4). The generalized eye structure of *Odontosyllis*, obtained from examining numerous microscopic sections (Figs. 3–10), was used to reconstruct the schematized eye in Figure 11.

It was also of interest to see if we could determine the nature of the visual pigment. Spectral analysis of the pink layer obtained from sucrose density fractionation showed two absorption peaks, one a sharp band in the neighborhood of 420–430 nm and another broader band around 500 nm. The spectral absorption peak around 500 nm could indicate that the visual pigment is a rhodopsin. However, our attempts to

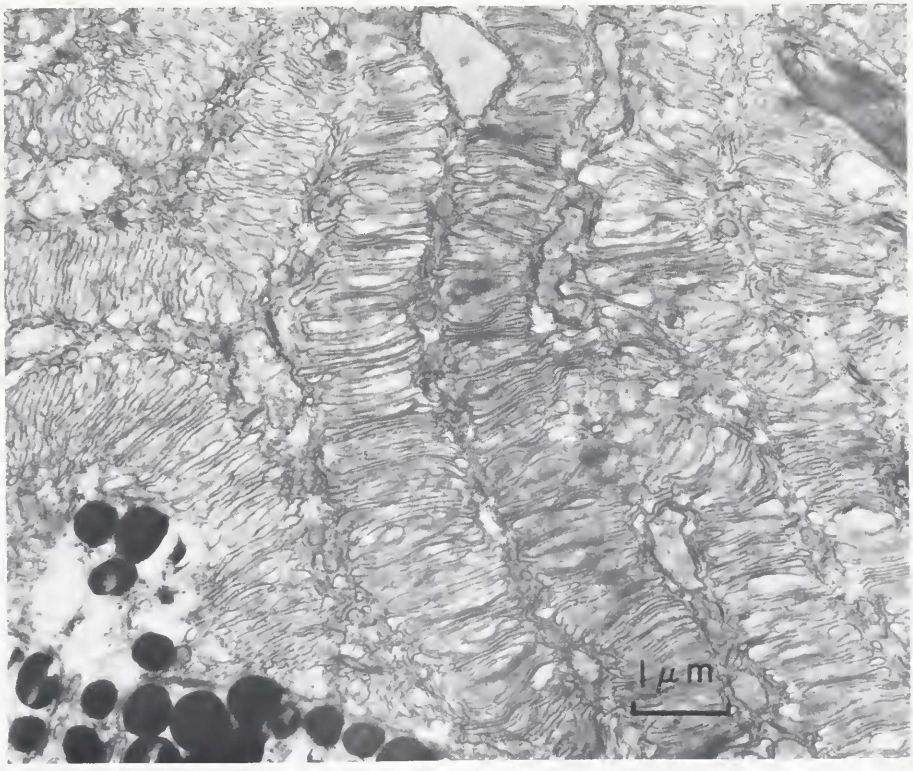


FIGURE 10. The retinal photoreceptors.

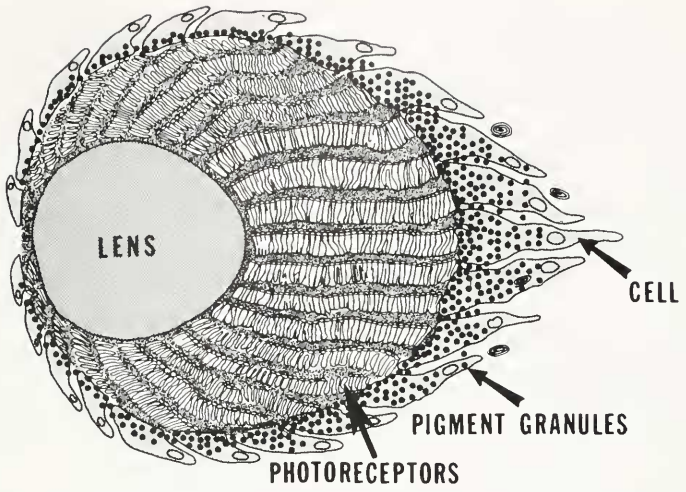


FIGURE 11. Schematic structure of the eye, showing lens photoreceptors, retinal cells, and pigment granules.

identify the visual pigment were limited due to the number of *Odontosyllis* heads (500) we could dissect and fractionate for analysis.

DISCUSSION

Considerable information has accumulated over the past century on annelid polychaetes, but their eye structure and visual physiology has not been extensively investigated, partly because it was believed until recently that they did not evolve image forming eyes and that many marine polychaetes of interest are rare and difficult to collect alive (Wald and Rayport, 1977).

As already noted, the electroretinogram (ERG) of the eyes of *Odontosyllis,* the visual spectrum, was found to coincide with the bioluminescent emission spectrum (Wilkens and Wolken, 1981). This indicated that the *Odontosyllis* eye detects the bioluminescent light signals. The questions we then raised were how is the eye structured for imaging and what is its visual pigment? We were also interested to learn how the eye structure differs from that of the alciopids that do possess image resolving eyes (Wald and Rayport, 1977).

In general, the structure of the eye of *Odontosyllis enopla* as we have described it, Figures 1–10, and schematized it in Figure 11, does not differ greatly from the description and early drawings of the eye by Galloway and Welch (1911). More recent studies of the eye by electron microscopy of a closely related species, *Odontosyllis ctenstoma,* found off the coast of Roscoff, France (Bocquet, 1977, 1983), indicate similarities to the mature *Odontosyllis enopla,* including the observation that the eye undergoes hypertrophy (Bocquet, 1977). The eye structure does not differ greatly from other polychaetes, for example *Platynereis dumerilii,* as studied by Fischer (1971). Of interest to us was that within the lens area there are rods (tubes) arranged in a linear array that appear to be aligned with the optical axis (Figs. 7–9). Such an arrangement for the lens could function as light guides to maximize the light collecting ability for the eye. If the rods behave in this fashion, the lens would possess a fiber-

optics system serving as an efficient light collector for the photoreceptors. Such a system would be consistent with the *Odontosyllis* environment, for successful mating depends upon the detection and location of the bioluminescent light. However, further studies of the optics of the eye beyond that of a light detecting system is necessary to resolve the question of how good an imaging system it possesses.

The retinal photoreceptor structure appears to be of rhabdomeric origin (Westfall, 1982). They are similar to the photoreceptors of arthropods, mollusc eyes, and vertebrate retinal rod outer segments (Wolken, 1971, 1975). In the alciopid *Vanadis* there is a unique accessory retina which is not found in *Torrea,* a surface worm. The additional retina for *Vanadis* was postulated by Wald and Rayport (1977) to act in conjunction with the main retina as a depth indicator (Waterman, 1974). This function is based on the differing rates of extinction with the depth of the wavelengths that penetrate the sea. The possession of two retinas permits greater sensitivity to the changing wavelengths of light. The anatomical arrangement of an accessory retina in these alciopid eyes appears to correspond with the multi-retinas found in deep-sea cephalopod mollusc and fish eyes. No such mechanism occurs in the eyes of *Odontosyllis,* which is consistent with its shallow water habitat. As to the nature of the visual pigment of *Odontosyllis,* spectral absorption indicates that it is a carotenoid, a rhodopsin system. However, a larger sample and further purification is necessary to identify more precisely the visual pigment.

The structure of the *Odontosyllis* eye as we visualize it could indicate that among the annelid polychaetes an evolutionary development occurred from that of a pinhole-type eye to that of a simple camera image-forming eye.

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LITERATURE CITED

ANDREWS, E. A. 1892. On the eyes of polychaetous annelids. *J. Morphol.* **7:** 169–222.

BOCQUET, M. 1977. Étude ultrastructure de l'organe photoréception d'*Odontosyllis ctenostoma* S/F: Eusyllinae (Annelid Polychaete). *J. Ultrastructure Res.* **58:** 210–217.

BOCQUET-VERGER, M. 1983. Les organes photorecepteurs des syllidiens (Annelids Polychetes). *L' Annee Biol.* **22:** 169–185.

FISHER, A. 1971. Der Einfluss des or—Gens auf die Pigmentierund bie *Platynereis dumerilii* (Polychaeta) I and II. *Wilhelm Roux' Arch.* **163:** 226–241 and 242–268.

GALLOWAY, T. W., AND P. S. WELCH. 1911. Studies on a phosphorescent Bermuda annelid, *Odontosyllis enopla* V. *Trans. Am. Microsc. Soc.* **30:** 13–39.

GREEF, R. 1877. Monograph alciopidae. *Nova Acta Leopold.* **39:** No. 2.

HERMANS, C. O., AND R. M. EAKIN. 1974. Fine Structure of the eyes of an alciopid polychaete, *Vanadis tangensis.* (Annelida). *Z. Morphol.,* Tiere **79:** 245–267.

HESSE, R. 1899. Untersuchungen uber die organe der lichtempfindung bie niederen thieren. V. Die augen den polychaten anneliden. *Z. Wiss. Zool.* **65:** 446–516.

HUNTSMAN, A. G. 1948. *Odontosyllis* at Bermuda and lunar periodicity. *J. Fish. Res. Board Can.* **7:** 363–369.

MARKERT, R. E., B. J. MARKERT, AND N. J. VENTREES. 1961. Lunar periodicity in spawning and luminescence in *Odontosyllis enopla. Ecology* **42:** 414–415.

NICOL, J. A. C. 1978. Bioluminescence in vision. P. 371 in *Bioluminescence in Action,* P. J. Herring, ed. Academic Press, New York.

SHIMOMURA, O., F. H. JOHNSON, AND Y. SAIGA. 1963. Partial purification and properties of the *Odontosyllis* luminescence system. *J. Cell. Comp. Physiol.* **61:** 275–292.

TRAINOR, G. L. 1979. *Studies on the* Odontosyllis *Bioluminescence System.* Ph.D. dissertation, Harvard University, Cambridge.

WALD, G., AND S. RAYPORT. 1977. Vision in annelid worms. *Science* **196:** 1434–1439.

WATERMAN, T. H. 1974. Underwater light and the orientation of animals. Pp. 415–443 in *Optical Aspects of Ocenaography,* N. G. Jerlov and E. S. Nielson, eds. Academic Press, New York.

WESTFALL, J. A., ed. 1982. *Visual Cells in Evolution.* Raven Press, New York. Pp. 91–105 and 107–136.

WILKENS, LON, A., AND J. J. WOLKEN. 1981. Electroretinograms from *Odontosyllis enopla* (polychaete; syllidae): Initial observations on the visual system of the bioluminescent fireworm of Bermuda. *Mar. Behav. Physiol.* **8:** 55–66.

WOLKEN, J. J. 1971. *Invertebrate Photoreceptors.* Academic Press, New York. Pp. 50–92.

WOLKEN, J. J., 1974. Comparative structure of invertebrate photoreceptors. Pp. 111–154 in *The Eye,* Vol. VI, H. Davson and L. T. Graham, Jr., eds. Academic Press, London.

WOLKEN, J. J. 1975. *Photoprocesses, Photoreceptors and Evolution.* Academic Press, New York. Pp. 137–190.

WOLKEN, J. J., AND R. G. FLORIDA, 1969. The eye structure and optical system of the crustacean copepod, *Copilia. J. Cell Biol.* **40:** 279–285.